Hypertonic Saline Dextran (HSD) in a Complex Military Injury – A Preclinical Study

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ABSTRACT

Introduction

Recent data from current conflicts indicate that a significant proportion of severely injured military casualties result from explosive events. Although these are evacuated to surgical care as quickly as possible, extended evacuation is sometimes enforced. Significant logistical and potential physiological advantage might be attained by employing low volume resuscitation with hypertonic intravenous fluids. Current military practice for initial far-forward resuscitation involves hypotensive resuscitation with isotonic crystalloids. We have reported, based on a porcine model of severe haemorrhage that prolonged hypotensive resuscitation causes substantial physiological deterioration. A hybrid strategy (using 0.9% saline) of initial hypotensive (60 min) followed by normotensive resuscitation offered significant physiological benefit after haemorrhage alone and significantly better survival after combined haemorrhage and blast injury.

Rationale

To examine the potential role of hypertonic saline dextran (HSD) in a hybrid hypotensive/normotensive resuscitation strategy after haemorrhagic shock alone and combined with blast injury.

Method

31 Large White pigs were terminally anaesthetised with alfaxalone (5-10 mg/kg/h). Two injury patterns were simulated:

1) *Haemorrhagic shock (controlled loss of 35% blood volume and uncompressed Grade IV liver injury);*
2) *Explosive injury (same haemorrhage/liver injury preceded by exposure to a blast shock wave resulting from the detonation of a bare explosive charge).*

Animals from each group were then resuscitated using the hybrid strategy (see above) where resuscitation was initiated with either HSD (7.5% saline / 6% dextran 70) or 0.9% saline (NS). The total amount of HSD was limited to 7.1 ml/kg and any subsequent resuscitation continued with 0.9% saline.

The protocol consisted of four groups: Group 1 (n=7) haemorrhage/HSD; Group 2 (n=11) blast/ haemorrhage/HSD; Group 3 (n=6) haemorrhage/NS; Group 4 (n=7) blast/haemorrhage/NS. The primary endpoint was survival up to 8h after the onset of resuscitation.

Results

Blast injury resulted in significant pulmonary compromise (PaO_2 8.3±0.3 & 8.7±0.5 Groups 2 & 4 vs 9.6±0.3 & 9.5±0.5 kPa Groups 1 & 3 mean±SEM). HSD was associated with a significantly reduced

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14. ABSTRACT
Recent data from current conflicts indicate that a significant proportion of severely injured military casualties result from explosive events. Although these are evacuated to surgical care as quickly as possible, extended evacuation is sometimes enforced. Significant logistical and potential physiological advantage might be attained by employing low volume resuscitation with hypertonic intravenous fluids. Current military practice for initial far-forward resuscitation involves hypotensive resuscitation with isotonic crystalloids. We have reported, based on a porcine model of severe haemorrhage that prolonged hypotensive resuscitation causes substantial physiological deterioration. A hybrid strategy (using 0.9% saline) of initial hypotensive (60 min) followed by normotensive resuscitation offered significant physiological benefit after haemorrhage alone and significantly better survival after combined haemorrhage and blast injury.

15. SUBJECT TERMS

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survival time (Kaplan-Meier survival analysis, Wilcoxon method) in the blast/haemorrhage groups (mean [95% CI], 208[77-339] vs 398[247-550] min respectively in Groups 2 & 4, P=0.04), but not in the groups subjected to haemorrhage alone (480[all survived] vs 448[393-502] min respectively in Groups 1 & 3, P=0.11). In the absence of blast injury HSD did confer some physiological advantage, with a reduced base deficit throughout the resuscitation period (e.g. arterial base excess after 60 min resuscitation -3.1±1.3 vs -9.0±3.1 mM Groups 1 & 3), but not after combined blast and haemorrhage (-13.9±1.4 vs -13.5±2.3 mM, Groups 2 & 4). In blast-injured animals given HSD (Group 2) the majority of early (<8h) deaths occurred within the first 60 min of resuscitation (5/7) in contrast to Group 4 where none of the early deaths were within the first 60 min.

Conclusions

Although HSD did show some physiological benefit after haemorrhage in the absence of blast injury, survival time was significantly shorter when animals were initially resuscitated with HSD after combined blast and haemorrhage. HSD should therefore be contraindicated when significant primary blast injury complicates haemorrhage.

1.0 INTRODUCTION

1.1 Introduction

Haemorrhage remains the leading cause of battlefield deaths [1] and the second leading cause of early death after civilian trauma [2]. Currently a high proportion of military battlefield injuries are the consequence of explosive events e.g. detonation of improvised explosive devices (IEDs) [3; 4] with recent reports indicating that 78-79% of casualties result from explosions. There are also instances of civilians being injured by explosives, with mass casualties resulting form terrorist attacks [5-8]. Once catastrophic haemorrhage has been arrested [9] fluid resuscitation is often needed to sustain life until the casualty is evacuated to surgical care. Military evacuation timelines can differ significantly from those normally found in civilian settings. Although evacuation times in mature military operations are predominantly short, as are now being seen in Afghanistan, timelines in less mature settings (as we have seen recently[10; 11]) can be considerably longer. Responsive resuscitation strategies such as the UK Battlefield Advanced Trauma Life Support (BATLS [12]) have therefore been developed to accommodate situations where evacuation timelines are extended and accounting for military injuries. In addition, these concepts may be applicable to civilian settings e.g. after terrorist bombings where there is often disruption to infrastructure and security issues relating to secondary devices resulting in delayed evacuation.

A key feature of far-forward fluid resuscitation of hypovolaemic casualties is the maintenance of an acceptable oxygen delivery to sustain life, and if possible to limit physiological deterioration while minimising the risk of disrupting nascent blood clots, causing re-bleeding as the casualty is evacuated to surgical care. A number of authorities advocate the use of 'hypotensive' resuscitation where fluid is limited or withheld to deliberately allow blood pressure to remain below normal levels and many military and civilian medical services now resuscitate casualties to a target systolic blood pressure of approximately 80 mm Hg (a palpable radial pulse in humans) [12; 13]. However, this approach is not without penalty since prolonged hypotension can result in poor tissue perfusion and ischemic damage [14; 15] and the clinical evidence supporting hypotensive resuscitation is based on relatively short evacuation times to surgical care e.g. 75 min reported by Bickell *et al* [16].

We have recently shown that when arterial oxygen content is reduced after primary blast lung injury, the additional reduction in tissue oxygen delivery during hypotensive resuscitation becomes unacceptably low and mortality increases rapidly after the first hour of resuscitation [15]. A compromise is therefore needed between several variables and has to evolve as the balance of risk changes. This is the basis of the current

BATLS strategy [12]. Initial resuscitation (for the first hour) is conducted to a hypotensive endpoint to minimize risk of blood clot disruption. During this time the clot begins to stabilise, attaining >80% of its ultimate tensile strength during the first hour and therefore becoming more resistant to disruption. If the casualty has not arrived at a surgical facility consideration is then given to limit the risk of death from physiological compromise, which has been developing during the first hour. Subject to a clinical evaluation of the balance of risk, after the first hour arterial blood pressure may be elevated (with additional fluid) to improve tissue blood flow before the effects of tissue ischaemia become overwhelming. However, by this time the clot has strengthened and is more likely to withstand the elevated blood pressure. This novel hybrid resuscitation strategy (NH) of initial hypotensive followed by normotensive resuscitation using 0.9% saline has been shown to significantly increase survival times (compared to prolonged hypotensive resuscitation) after combined primary blast injury and controlled/uncontrolled haemorrhage (simulating secondary blast injury) [17]. Although there was no apparent survival advantage with the NH strategy after haemorrhage in the absence of blast injury, since survival was already high with hypotensive resuscitation, NH did result in very significant physiological advantage by reversing a clinically significant metabolic acidosis [17]. There was no evidence of increased secondary blood loss with NH compared to hypotensive resuscitation in the model used which incorporated an uncompressed Grade IV liver injury [17].

The initial demonstration of NH resuscitation utilised 0.9% saline as the only resuscitation fluid. It is possible that further advantage could be gained by initiating resuscitation with a hypertonic solution e.g. hypertonic saline dextran (HSD). Hypertonic solutions have been advocated for far forward military resuscitation [18] since they possess a significant logistical benefit in terms of reduced weight burden; 250 mL of HSD is reputed to be equivalent to 3 L of 0.9% saline with respect to early plasma volume expansion. Hypertonic solutions are also known to dampen the systemic inflammatory response that develops after trauma and which is thought to be part of the aetiology underlying later complications [19-22]. This benefit of HSD has been shown in a number of clinical studies [21-23]. In addition, hypertonic solutions have been shown to confer microcirculatory benefit, possibly improving the distribution of blood flow in tissue [24]. Consistent with this, in an early study we found that HSD reduced the metabolic acidosis during hypotensive resuscitation after haemorrhage alone [25]. Unfortunately this beneficial effect was not apparent after combined blast injury and haemorrhage, possibly because the microcirculatory effect was simply not sufficient to overcome the very poor tissue oxygen delivery due to combined low arterial oxygen content (due to blast lung) and low flow (due to the hypotension) [25]. However, with a resuscitation strategy that promotes better tissue perfusion the beneficial effects of HSD may be apparent after combined blast injury and haemorrhage.

The aim of the present study was to determine whether HSD, as part of a resuscitation strategy employing the NH blood pressure profile (initial hypotensive followed by normotensive resuscitation), is at least as effective as 0.9% saline in relation to survival. Furthermore, we sought to determine whether HSD was superior to 0.9% saline with respect to physiological changes by limiting the initial deterioration in acid base status during the hypotensive phase of resuscitation and enhance the acid base improvement during the normotensive phase.

2.0 METHODS

The study was conducted on terminally anaesthetized crossbred Large White pigs (41-57 kg) and was ethically reviewed and conducted in accordance with the Animals (Scientific Procedures) Act, 1986. The animals were housed indoors and were fed on a complete wheat-soya based ration at 1.5–1.7 kg per day. They were allowed water *ad libitum.*

2.1 Surgical Preparation

The animals were fasted for 18 hours before the surgical procedure, but allowed water *ad libitum*. After pre-medication with intramuscular midazolam hydrochloride (0.1 mg/kg) anaesthesia was induced by mask with isoflurane (5%) in a mixture of oxygen and nitrous oxide (1:1) and the animals intubated. Surgical anaesthesia was subsequently maintained with isoflurane (1–2 %) in a mixture of oxygen and nitrous oxide (1:2). Initial monitoring consisted of end-tidal CO_2, pulse oximetry via a tail probe and skin surface electrocardiogram electrodes (Propac 106EL, Protocol Systems Inc., Oregon). With the animal positioned supine surgical preparation took place after skin preparation with povidone-iodine solution (10% w/v, Betadine Aqueous Antiseptic Solution, Seaton Healthcare Group plc, UK).

The left carotid artery, both internal jugular veins, left femoral artery and vein were cannulated (Portex 8FG, Hythe, UK). A balloon tipped flow-directed cannula (744MF75 Swan Ganz, Edwards Life Sciences Ltd, Newbury, UK) was introduced via a right internal jugular vein cannula introducer sheath, (Desivalve Catheter Introducer, Vygon, Cirencester, UK) and advanced until its tip was in the pulmonary artery. Cannula placement was determined by monitoring pressure changes at the tip.

Once venous access had been established anaesthesia was maintained with intravenous alphaxolone (Alphaxan, Vetoquinol UK Ltd, UK) and the isoflurane discontinued.

A midline laparotomy was performed, the spleen contracted by topical application of adrenaline (up to 1.5 ml of a 1 $mg.ml^{-1}$ solution) before removal. A surgical snare was inserted into the left medial lobe of the liver for later induction of a Grade IV liver injury, and the snare exteriorized via the laparotomy. The bladder was catheterized by open suprapubic cystostomy. All incisions were closed *en masse*. Animals were allowed to breathe spontaneously for the remainder of the experiment unless they displayed marked respiratory depression, at which stage Synchronized Intermittent Mandatory Ventilation (SIMV) was instituted in an attempt to maintain adequate oxygenation and prevent severe hypercapnoea. The animals recovered from surgery under anaesthesia for one hour before baseline measurements were made during which time they were transported to the physiological monitoring suite near the explosives arena.

2.2 Cardiovascular Monitoring

Arterial blood pressure was recorded via the carotid artery cannula and pulmonary arterial and central venous pressures were recorded via the flow-directed balloon-tipped flotation catheter. Physiological pressure measurements were made using strain gauge manometers (Sensonor 840, SensoNor a.s., Norway) and zero pressure for all transducers was set at heart level. Body temperature was maintained at approximately 38°C using external heating/cooling and blankets as appropriate. The bladder was drained at hourly intervals.

All cardiovascular variables were recorded using a computerized data acquisition system (Maclab 8/s, ADInstruments, UK) and associated software (Chart v4.2.3, ADInstruments, UK) for subsequent analysis.

2.3 Blood Gas and Related Chemistry

Arterial and venous blood samples were taken anaerobically into heparinised syringes from the carotid and pulmonary artery catheters respectively for blood gas, base excess and lactate analysis (Gem Premier 3000 Blood Gas Analyzer, Instrumentation Laboratories, Warrington, UK).

2.4 Experimental Protocol

The development of the baseline model of primary blast injury and haemorrhage used in this study is described in detail elsewhere [26]. This model has been further adapted for the present study by the

addition of the liver snare and later induction of a Grade IV liver injury to allow an incompressible haemorrhage and the capacity to re-bleed during resuscitation. The animals were randomly allocated to one of four groups at the outset: Group 1 (n=7) haemorrhage/HSD; Group 2 (n=11) blast/haemorrhage/HSD; Group 3 (n=6) haemorrhage/NS; Group 4 (n=7) blast/haemorrhage/NS where the initial resuscitation fluids were either hypertonic saline dextran (HSD) or 0.9% saline (NS). The protocol is summarised in Figure 1. One hour after the end of surgery three cardiovascular measurements were made 5 minutes apart and paired arterial and mixed venous blood gas samples taken at the time of the first and third baseline cardiovascular measurement. After the baseline measurement the animals were moved outdoors, wrapped in a Kevlar blanket to protect from secondary and tertiary blast effects and positioned on a trolley 2.15 m from a cylindrical charge of EDC1S explosive (2.2 kg) which was then detonated remotely. Animals subjected to sham blast were treated identically but not exposed to blast.

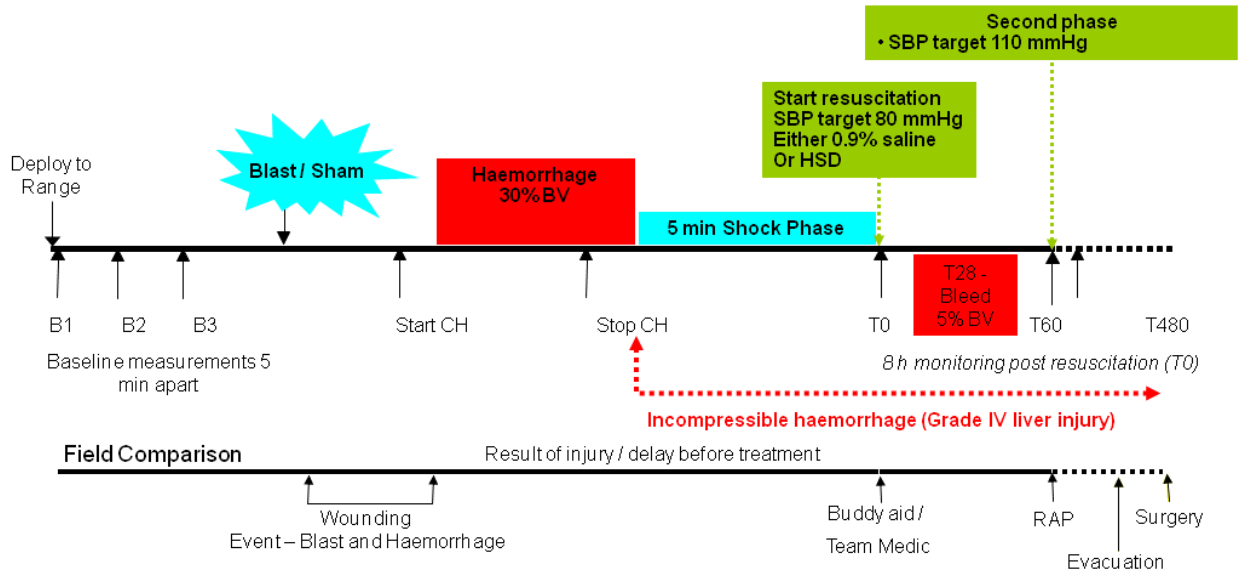

Figure 1: Experimental protocol which commenced approximately 1 hour after the end of surgery. CH, controlled hemorrhage; BV, total estimated blood volume; SBP, systolic arterial blood pressure.

Immediately after the blast (or sham blast) the animal was returned to the physiological monitoring suite and twenty minutes later, all animals were subjected to a controlled haemorrhage of 30% of their estimated total blood volume (B_0; Equation 1) over 4 minutes via the femoral arterial cannula, using a computer-controlled pump (Masterflex® L/S® model 7550-17, Cole Palmer Instrument Company, Chicago, IL). The rate of bleeding reduced exponentially as the haemorrhage progressed (Equation 2) to mimic the rate of haemorrhage from a major arterial lesion.

$$B_0 = 161.4751(W^{-0.2197})$$

Equation 1 Equation used to estimate total blood volume.[27] B_0 = total blood volume (ml.kg^{-1}) and W = body weight (kg).

$$V = B_0(1\text{-}e^{-0.04t})$$

Equation 2 Rate of blood loss during controlled haemorrhage.[28] V, total blood loss at time t in ml.kg^{-1}; B_0, initial blood volume in ml.kg^{-1}; t, % time until death; B_0, initial blood volume.

Following haemorrhage, the animals underwent a 5-min shock period where no treatment was administered before commencement of fluid resuscitation (time = T_0) (Figure 1).

Both resuscitation protocols utilized an identical blood pressure profile based on the novel hybrid approach; hypotensive resuscitation (target systolic arterial blood pressure, SBP, 80 mmHg) for the first hour and thereafter a normotensive target (SBP 110 mmHg). All animals were subjected to a simulated re-bleeding episode by controlled removal of 5% total estimated blood volume 28 min into the resuscitation phase. The difference between resuscitation strategies resides in the initial fluid used for resuscitation. The control groups were resuscitated with 0.9% saline (3 mL/kg/min) throughout. In the HSD groups resuscitation was initiated with 7.5% hypertonic saline / 6% dextran (HSD, Rescue Flow, BioPhausia, Stockholm, Sweden) given in aliquots of 0.71 mL/kg at 1 mL/min/kg, with a lockout period of 2 min between doses to avoid rapid increases in arterial blood pressure and breaching of the target SBP. The total amount of HSD was capped at 7.1 mL/kg (500 mL/70kg being the maximum recommended dose for military casualties [29]). Thereafter resuscitation continued with 0.9% saline as in the control group. In all groups aliquots of 0.9% saline or HSD were given as necessary to attain and maintain the target SBP.

Resuscitation fluid was administered according to the relevant protocol for 8 hours or until the animal died, if sooner. Cardiovascular and blood gas measurements were made before and after haemorrhage, at T_0 and then at 15 min intervals until 60 min after the onset of resuscitation (T_{60}) and thereafter at 30 min intervals for the remainder of the study. Arterial and mixed venous blood samples for blood gas analysis were taken at the same time points as the cardiovascular measurements until T_{120} and hourly intervals thereafter.

2.5 Primary Endpoint of the Study and Post-Mortem Assessments

The primary end point of the study was survival time (time until death) with death defined as a pulse pressure of 0 mmHg. After attainment of the primary endpoint or survival to T_{480}, a lethal overdose of sodium pentobarbitone (Euthatal, Merial Animal Health Ltd, Harlow, Essex) was administered intravenously. The lungs were removed, areas of contusion noted and the lungs weighed for calculation of the lung weight index (lung weight/body weight).

2.6 Statistical Analysis

All data are presented as mean±s.e.mean unless indicated otherwise. Survival times were compared using Kaplan-Meier survival analysis (Wilcoxon method) and the data stratified with respect to blast or sham blast injury and analyzed using Minitab (v14). Data from animals still alive after 8 hours were treated as right-censored. Cardiovascular, blood gas and chemistry data were compared using two-way analysis of variance (ANOVA) with repeated measures over time. Single time-point analyses were made using 2 way and 1 way ANOVA as appropriate. In all cases a significance level of $P \leq 0.05$ (two tailed) was used.

3.0 RESULTS

Baseline (pre-injury) values are shown in Table 1. There were no significant differences between groups in the initial parameters (one way ANOVA). Body temperature did not change significantly during the course of the study (two-way ANOVA with repeated measures for time).

Table 1: Initial values of physiological data and body weights.

	Group 1	Group 2	Group 3	Group 4
Body wt (kg)	52.3±0.9	49.3±0.9	47.2±1.8	49.3±1.4
Temp (°C)	38.7±0.2	38.3±0.2	37.5±0.2	38.8±0.3

Blast injury resulted in significant respiratory compromise (PaO2 6.3±0.8 & 5.6±0.6 Groups 2 & 4 vs 9.5±0.4 & 9.6±0.5 kPa Groups 1 & 3) 2 min after blast exposure. At this time the blast-injured animals were also hypercapnoeic (PaCO2 6.9±0.2 & 7.2±0.5 Groups 2 & 4 vs 5.9±0.2 & 6.3±0.3 kPa Groups 1 & 3) consistent with lung compromise and/or hypoventilation. Twenty minutes after blast there had been a partial resolution (PaO2 8.3±0.3 & 8.7±0.5 Groups 2 & 4 vs 9.6±0.3 & 9.5±0.5 kPa Groups 1 & 3) and PaCO2 had returned to normal levels (Figure 2), suggesting approximately normal respiration but some lung compromise.

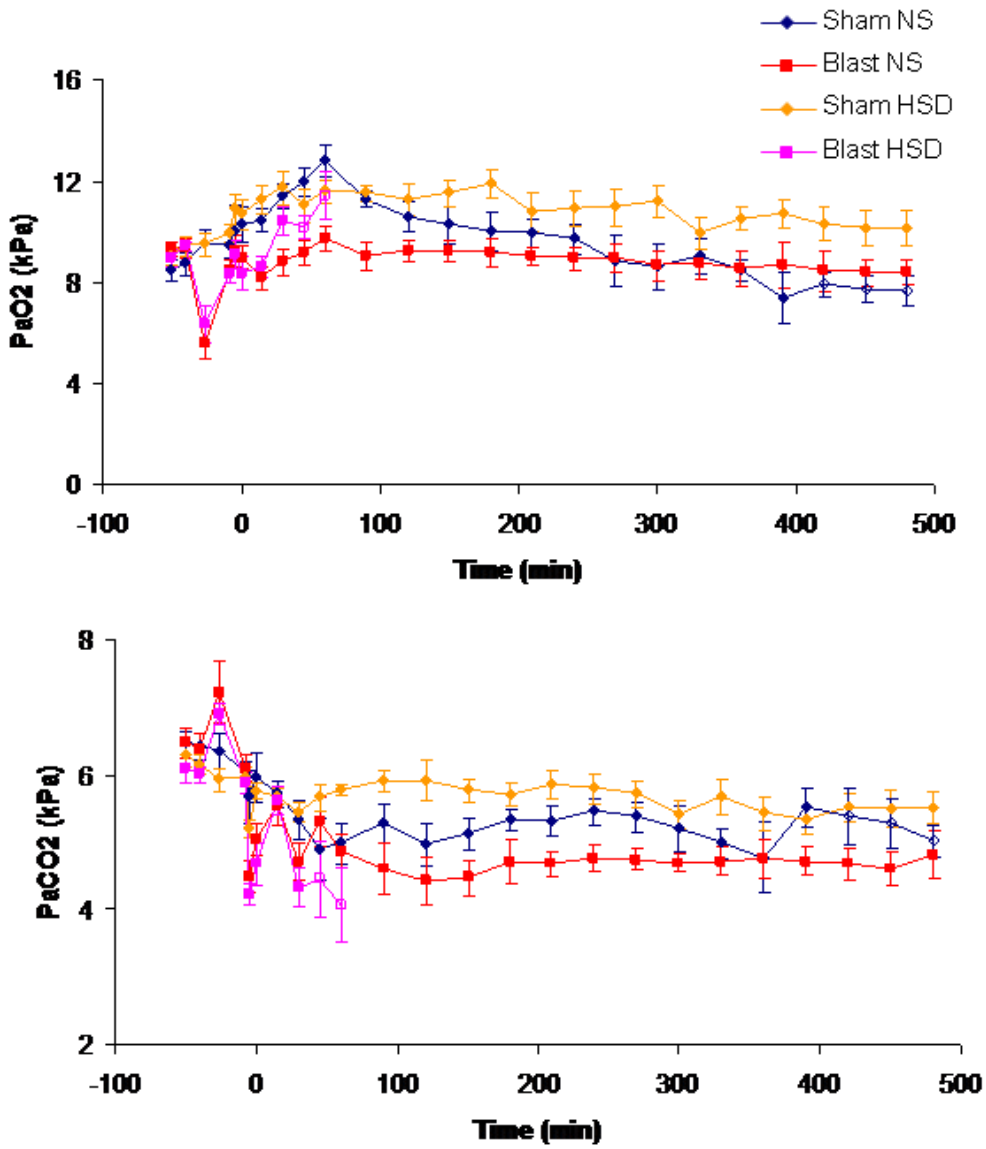

Figure 2: Arterial oxygen tension (PaO_2) and carbon dioxide tension ($PaCO_2$) in four groups of animals subjected to either sham blast (S) or blast (B), hemorrhagic shock and resuscitation initiated with either hypertonic saline dextran (HSD) or 0.9% saline (NS). Mean values ± SEM.

3.1 Effect of HSD on Survival

HSD was associated with a significantly reduced survival time (Kaplan-Meier survival analysis, Wilcoxon method, Figure 3) in the blast/haemorrhage groups (mean [95% CI], 208[77-339] *vs* 398[247-550] min

respectively in Groups 2 & 4, P=0.04), but not in the groups subjected to haemorrhage alone (480[all survived] vs 448[393-502] min respectively in Groups 1 & 3, P=0.11).

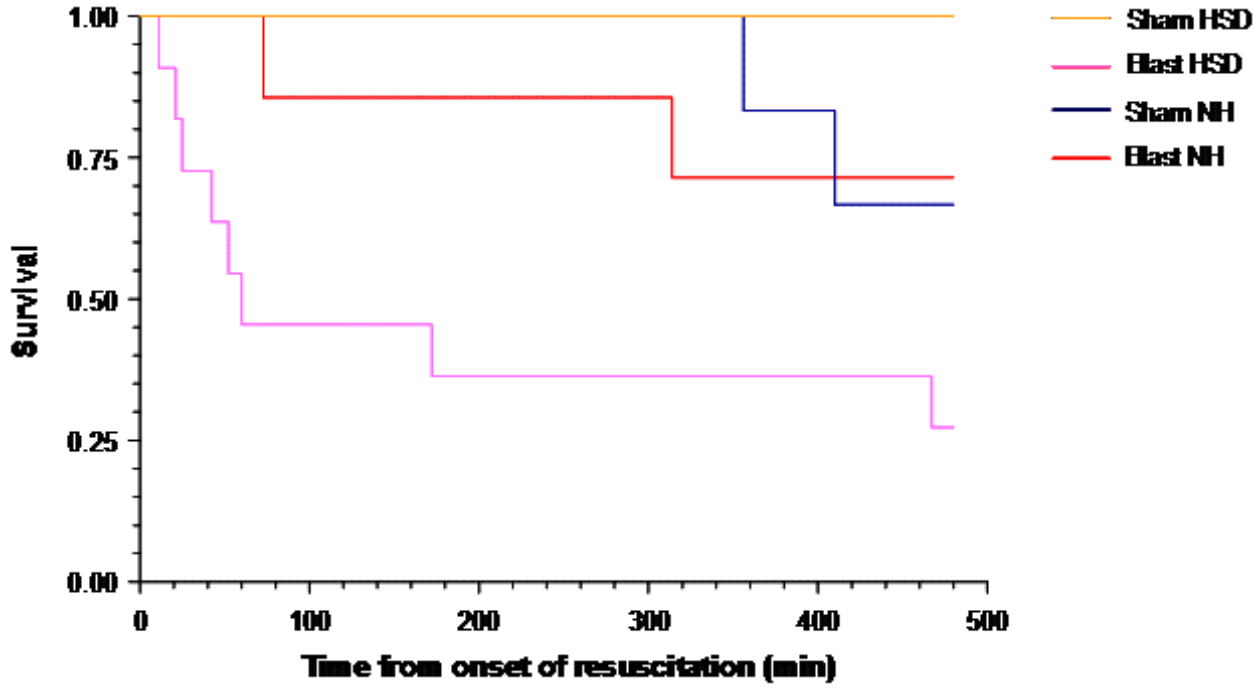

Figure 3: Kaplan-Meier survival plot for four groups of animals subjected to either sham blast (S) or blast (B), hemorrhagic shock and resuscitation to initiated with either hypertonic saline dextran (HSD) or 0.9% saline (NS).

3.2 Patterns of Response to HSD

Poor survival in the group subjected to combined blast injury and haemorrhage and subsequently resuscitation with HSD was due to poor responsiveness to HSD. An example of a 'good' response to HSD is given in Figure 4a,b. HSD can be seen to cause and initial, brief, fall in arterial blood pressure followed by a significant rise. In both these cases two doses were required to attain the target SBP. The individual animals shown in Figure 4a,b continued to respond well to HSD and survived, respectively for 480 any 467 min after onset of resuscitation. By contrast, an example of 'poor' response to HSD is shown in Figure 4c. This animal showed little response to HSD, the target SBP was not met despite repeated doses of HSD and the animal eventually succumbed within 21 min of the onset of resuscitation. A more dramatic HSD failure is shown in Figure 4(di and dii). Here the response to HSD was initially good and the target SBP was attained. However, this was followed by a sudden collapse and death approximately 15 min after the onset of resuscitation, despite further attempts at fluid resuscitation as directed by the protocol. Of 11 animals in Group 2 (combined blast and haemorrhage, resuscitated with HSD), 6 showed a good response to HSD and survived beyond the first hour of the study, 3 failed to respond (did not attain the target SBP) and 2 showed the catastrophic collapse shown in Figure 4d. Furthermore, one of the animals that failed to attain the target SBP also showed a sudden collapse in blood pressure prior to death. In the absence of blast injury all animals given HSD responded well and survived the duration of the study (8 hours from onset of resuscitation).

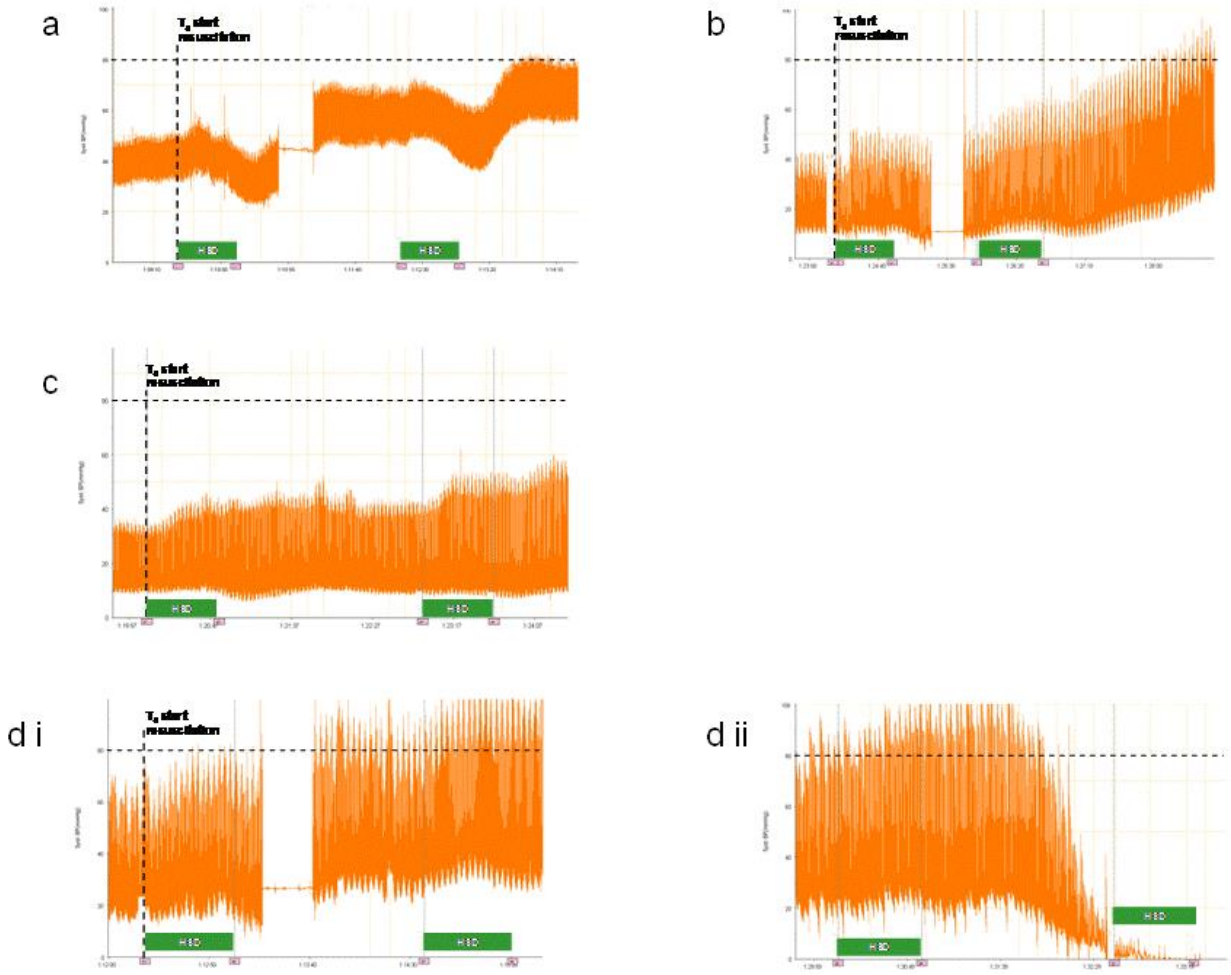

Figure 4: Systemic arterial blood pressure response to infusion of hypertonic saline / dextran (HSD) in four individual animals. All animals had received a controlled haemorrhage followed by a Grade IV liver injury. Preceding the haemorrhage animals were either subject to blast injury or no blast injury as follows: a) no blast injury, good response to HSD; b) blast injury, good response to HSD; c) blast injury, poor response to HSD, target blood pressure was not attained before animal died; d1) blast injury, initial good response to HSD and attainment of target blood pressure followed approximately 15 min later by dii) catastrophic collapse and death. Blocks labelled HSD indicate periods of HSD infusion.

3.3 Blood Chemistry and Oxygen Transport

There was a significant increase in plasma sodium levels associated with the onset of resuscitation with HSD in both blast and sham blast-injured groups (Figure 5). This elevation in plasma sodium associated with HSD persisted until the end of the study (Figure 5). There was no significant effect of injury (blast vs no blast) on plasma sodium. Examination of data from individual animals (Figure 6) confirms this pattern and dos not reveal any dramatic differences between HSD responders and non-responders (those that failed to survive the first hour of resuscitation). The highest plasma sodium level was seen in animal BHSD9 immediately prior to death, however, this level was not excessive compared to other animals which did respond and survived considerably longer periods.

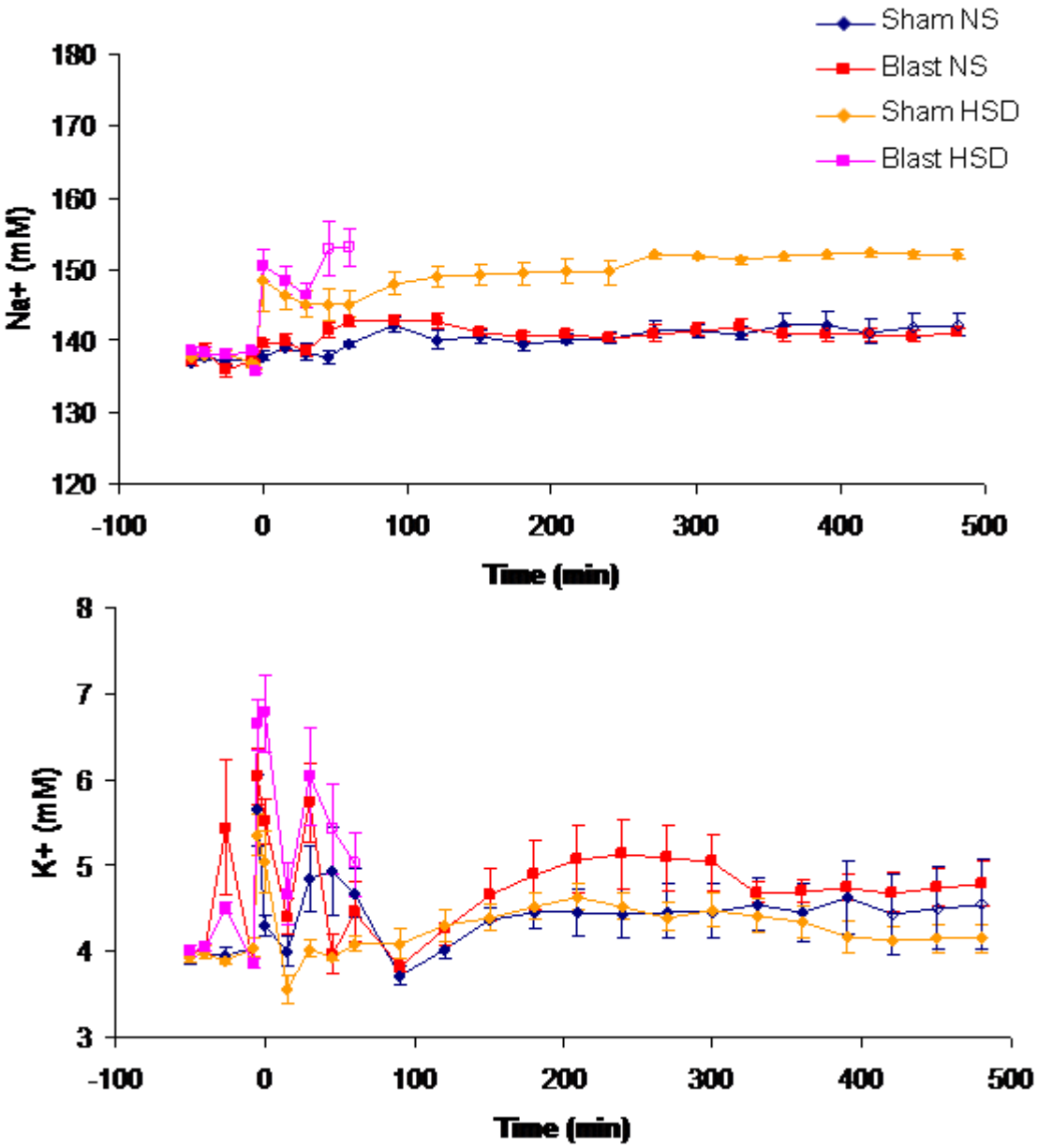

Figure 5: Arterial plasma sodium (Na+) and potassium (K+) concentrations in four groups of animals (for further details of groups see legend to Figure 2). Mean values ± SEM.

Plasma potassium showed a transient, significant, increase associated with blast injury (Figure 5), which resolved over the following 15 min so that there was no significant difference between groups by the onset of haemorrhage (Figure 5). All groups showed a significant increase after haemorrhage, with the highest level being seen in the blast injured groups, although this difference was not statistically significant. The elevated potassium levels gradually resolved over the first 100 min of resuscitation before showing a secondary rise in the surviving blast group (resuscitated with 0.9% saline). It is impossible to comment of the blast-injured group resuscitated with HSD since the majority did not survive to these latter time points.

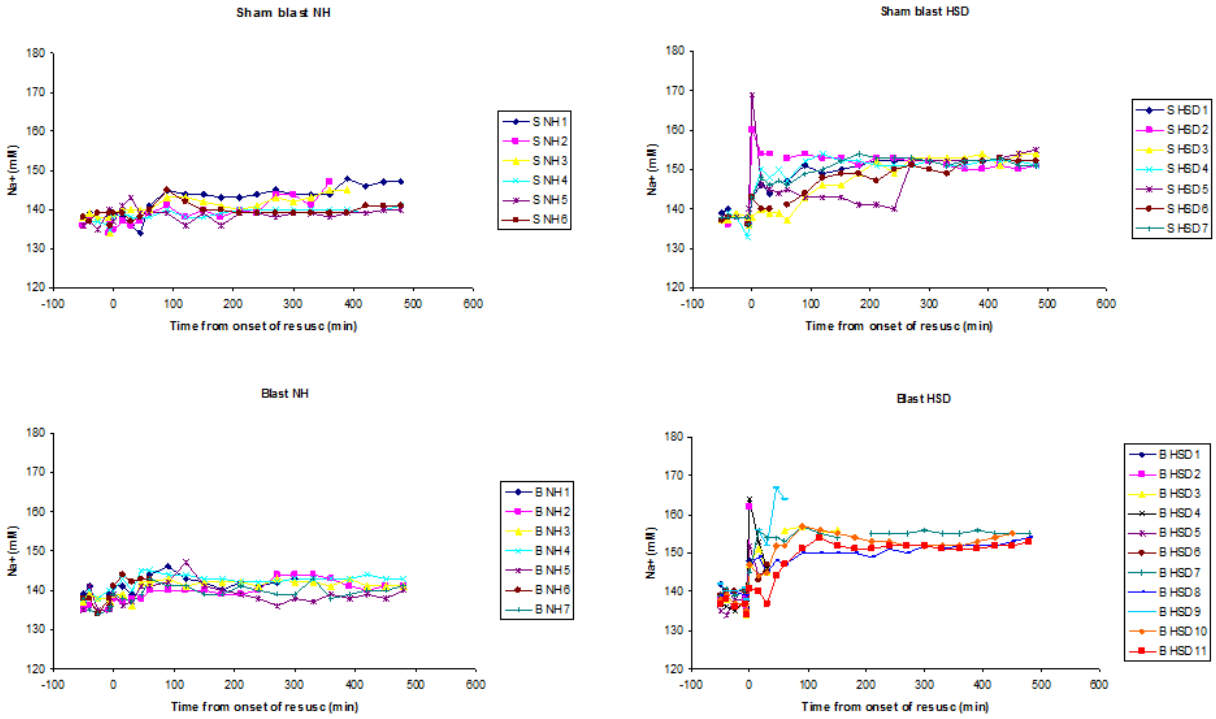

Figure 6: Arterial plasma sodium (Na+) from individual animals in each of the four groups (for further details see legend to Figure 2).

There was a significant fall in haematocrit in all groups during resuscitation. However, there was no significant difference between injury type (blast / no blast) or resuscitation fluid (0.9% saline / HSD) (Figure 7). Examination of data from individual animals (Figure 8) did not reveal any clear differences between those that responded to HSD and those that did not. A sharp fall in haematocrit was seen in BHSD1, 4 and 9 associated with early death. However, this represents the normal pattern of response to HSD that mobilises interstitial water into the plasma compartment. This finding eliminates one potential mechanism of HSD failure, namely inability to expand plasma volume.

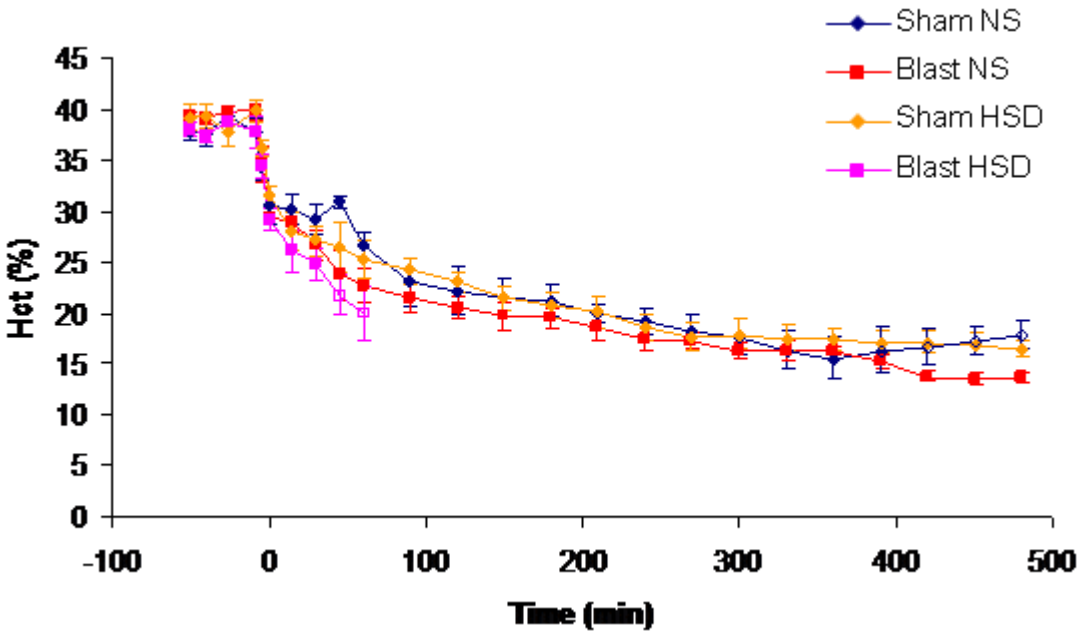

Figure 7: Arterial haematocrit (Hct%) in four groups of animals (for further details of groups see legend to Figure 2). Mean values ± SEM.

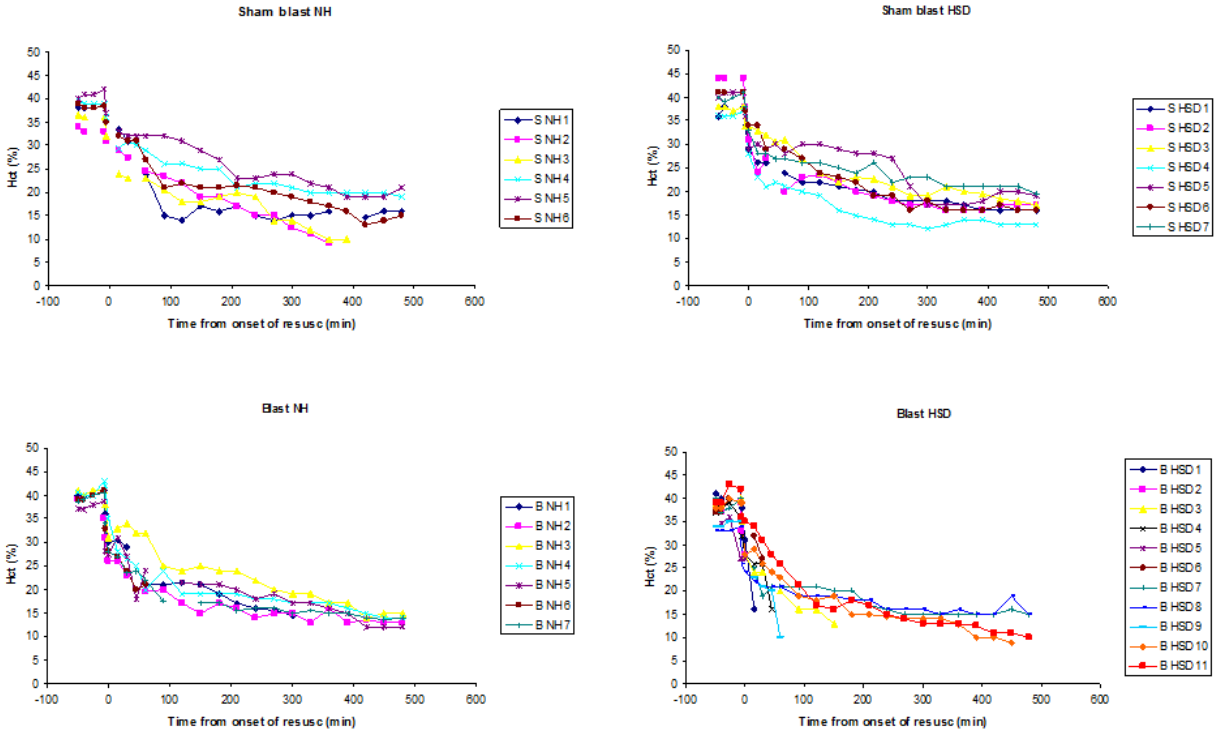

Figure 8: Arterial haematocrit (Hct%) from individual animals in each of the four groups (for further details see legend to Figure 2).

Coincident with the resuscitation and the fall in haematocrit there was a significant reduction in arterial oxygen content in all groups (Figure 9). The reduction was greatest in the animals subjected to blast injury

since this group experienced both haemodilution and a fall in arterial oxygenation due to blast lung. Oxygen extraction ratio (OER) increased significantly in all groups by the end of the shock period. The increase was greatest in the groups subjected to blast injury, which showed a significantly higher OER during resuscitation. Attainment of the higher resuscitation target blood pressure 60 min after the onset of resuscitation resulted in a significant reduction in OER in all surviving groups (Blast NH, Sham blast NH and Sham blast HSD), although the OER remained significantly higher in the Blast NH group compared to Sham blast. There was no significant effect of resuscitation fluid (0.9% saline vs HSD) on OER (Figure 9).

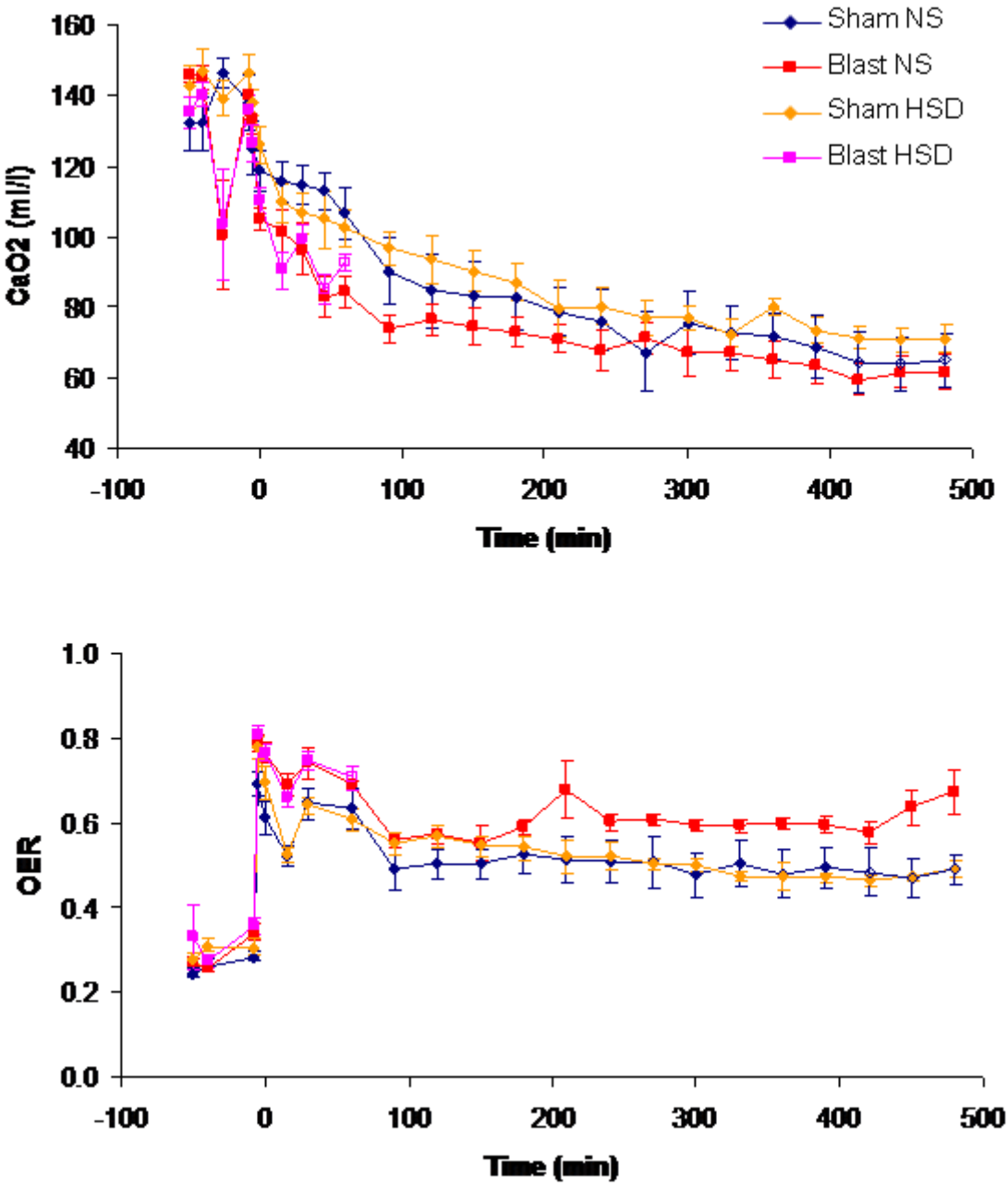

Figure 9: Arterial oxygen content (CaO_2) and oxygen extraction ratio (OER) in four groups of animals (for further details of groups see legend to Figure 2). Mean values ± SEM.

Finally, during the hypotensive phase of resuscitation there was a significant fall in arterial base excess (ABE, Figure 10). Blast injury was associated with a significantly greater reduction in ABE (more profound metabolic acidosis) compared to sham blast (Figure 10). The metabolic acidosis was reversed in all surviving groups after the onset of normotensive resuscitation (Figure 10). During this normotensive phase (60-480 min after onset of resuscitation) there was a significant difference between 0.9% saline and HSD in the sham blast groups, with the animals resuscitated initially with HSD experiencing the least degree of metabolic acidosis (Figure 10). A similar comparison for the blast-injured groups could not be made due to the early demise of animals in the Blast/HSD group.

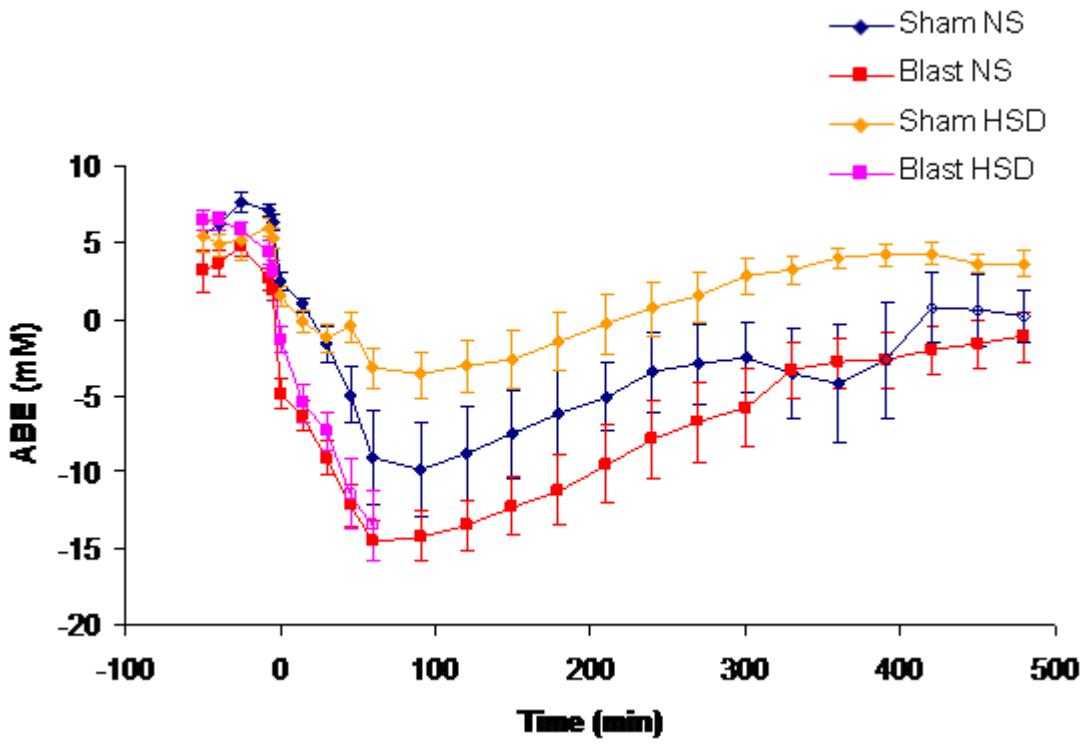

Figure 10: Arterial base excess (ABE) in four groups of animals (for further details of groups see legend to Figure 2). Mean values ± SEM.

3.4 Volume of Uncontrolled Haemorrhage

The volumes of intra-abdominal fluid, normalised for survival time, are shown in Figure 11. There were no significant differences between groups in the volume of intra-abdominal fluid found at post-mortem. The only animals that are clearly different from the overall pattern are three animals that died early during resuscitation with HSD. The absolute volume of intra-abdominal fluid in these animals was 97, 126 and 181 mL respectively (reported in ascending order of survival time).

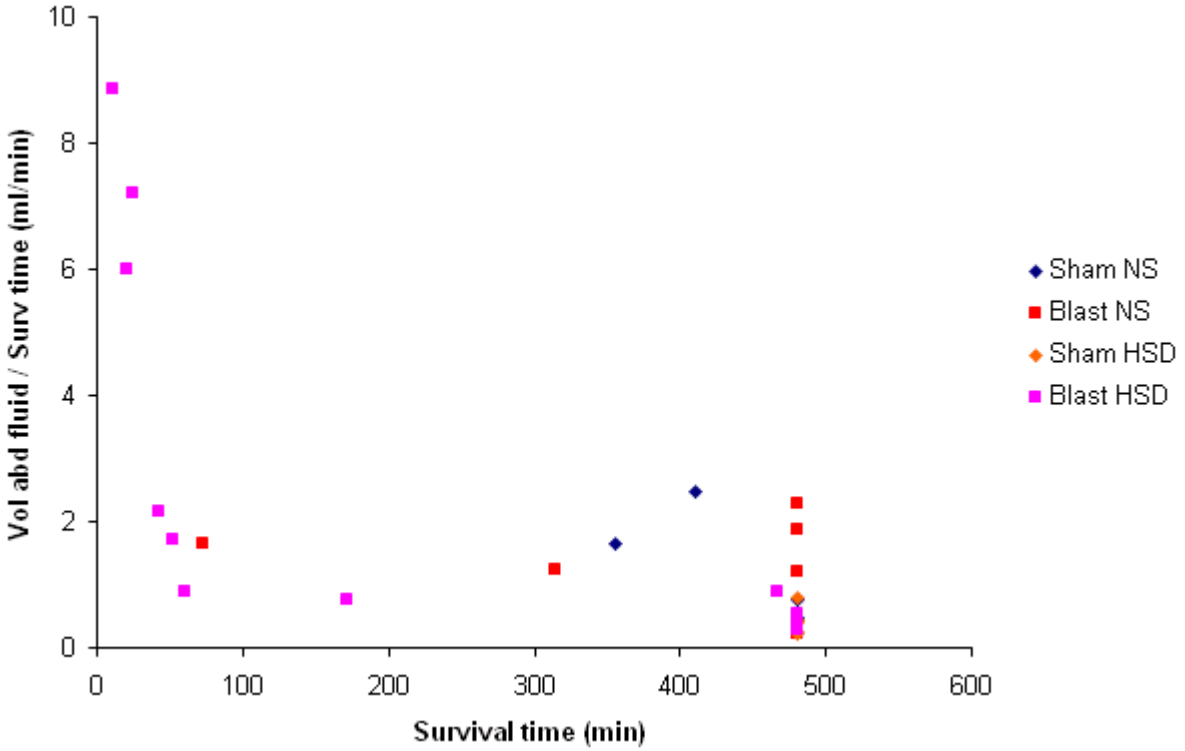

Figure 11: Volume of intra-abdominal fluid assessed at post-mortem in individual animals from each of four groups (for details of groups see legend to Figure 2). Fluid volumes are normalised for survival time.

4.0 DISCUSSION

The principal finding of this study is that HSD was significantly inferior to 0.9% saline (with respect to survival) when used to initiate a hybrid hypotensive/normotensive resuscitation strategy after combined primary blast injury and haemorrhage. A combination of primary blast injury and haemorrhage simulates a casualty injured in an explosion and suffering primary blast injury (e.g. blast lung) and secondary blast injury due to fragments resulting in blood loss. By contrast, survival when HSD was used to initiate resuscitation after haemorrhage alone (in the absence of primary blast injury) was at least as good as that seen with 0.9% saline. Furthermore, the physiological response to resuscitation with HSD after haemorrhage alone was significantly better than that seen with 0.9% saline. Unfortunately, since it would be very difficult (if not impossible) to quantify the extent of primary blast injury in a pre-hospital battlefield casualty initiating resuscitation with HSD in casualties from explosive events would be unsafe. Because military strategies often require a 'one size fits all' approach with respect to pre-hospital fluid resuscitation HSD is unlikely to be of utility for routine pre-hospital management of battlefield casualties.

The clinical significance of primary blast injury is very dependent on the circumstances surrounding injury. Casualties most likely to suffer primary blast injury are those exposed to blast in confined spaces or injured by enhanced blast weapons. When a conventional munition is detonated in an open environment the principal threat is fragments since the shock wave (responsible for primary injury) initially diminishes by an inverse cube function of distance from the explosion. However, the likelihood of primary blast injury increases even in an open environment if the casualty is near a reflecting surface. Fragments from the casing of a 'typical' munition, e.g. mortar shell, can be projected with lethal energy for much greater distances than the shock wave responsible for primary blast injury. Although it is very important to note that lethality due to fragments (secondary blast injury) is dependent on the probability of

being hit (which diminishes with distance away from the explosion as the fragments spread out) and the body structure damaged, it is true to say that the greatest threat to life with an 'outdoor' explosion is secondary blast injury. However, when a blast occurs in a confined space with multiple reflecting surfaces then primary blast injury assumes a much greater clinical significance [6; 8; 30].

The mechanism(s) whereby HSD fails after combined primary blast injury and haemorrhage is of particular interest. Unfortunately, although the data presented here gives some clear exclusions of potential mechanisms it does not identify a clear mechanism of failure. Three plausible possibilities can be excluded based on the current data: catastrophic re-bleeding, increased vascular permeability attenuating the ability of HSD to expand plasma volume and acute sodium toxicity.

An immediate concern with the use of HSD in early resuscitation after haemorrhage is that it could cause a significant over-shoot of arterial blood pressure and hence disrupt a fragile nascent blood clot, resulting in fatal re-bleeding. This is a particular concern of those advocating hypotensive resuscitation [16; 28; 31; 32] and certainly is legitimate in the early stages of resuscitation before blood clots have gained sufficient tensile strength to withstand elevations of arterial blood pressure towards normotensive levels or beyond. In the present study we were very careful to control the rise of blood pressure by administering the HSD in small aliquots. In cases where HSD was initially effective we generally attained the hypotensive target systolic arterial pressure (SBP, 80 mmHg) during initial resuscitation after 2-3 doses of HSD, representing 20-30% of the maximum recommended amount (500 mL / 70 kg [29]). Although in some circumstances this did result in a transient increase in SBP above the target by approximately 10 mmHg it did not result in re-bleeding from the uncompressed Grade IV liver injury. Although examination of Figure 11 indicates that the three animals surviving the shortest period after HSD administration did have the highest normalised (by survival time) volume of intra-abdominal fluid, this equated to 97-181 mL of absolute terms. These volumes are very unlikely to have contributed to the failure of HSD and therefore re-bleeding can be excluded as a mechanism of early death after HSD (HSD-failure) in the current study. However, these data highlight another potential problem with pre-hospital use of HSD in austere environments. We were able to accurately control the rise in blood pressure during HSD administration by titrating small aliquots, guided by the use of continuous blood pressure monitoring. In pre-hospital clinical use under difficult circumstances (where the caregiver themselves may be under considerable threat) the HSD may be given slowly one 'bag' (250 mL) at a time (approximately 50% of the maximum recommended dose) without accurate blood pressure monitoring. There is therefore a significant likelihood that this might breach the guidance of initial hypotensive resuscitation and could result in re-bleeding.

A second potential mechanism underlying the failure of HSD after primary blast injury and haemorrhage relates to microvascular permeability. Should microvascular permeability be increased in this circumstance then the osmotic effect of HSD would be reduced and its ability to quickly increase intravascular volume by redistribution of interstitial fluid would be attenuated. This mechanism has precedence in casualties with brain injury where hypertonic solutions can sometimes have limited effect (Dimitris, personal communication). This is clearly not the case in the present study. Examination of Figure 8 shows that all animals treated with HSD showed a fall in haematocrit which, in part, was due to the hypertonic solution mobilising interstitial water into the vascular space and hence causing haemodilution. Failure of this mechanism would result in an attenuated fall in haematocrit. However, Figure 8 does not reveal that any of the early deaths was associated with an attenuated reduction in haematocrit, thus eliminating this potential mechanism.

The third possibility underlying the failure of HSD is acute sodium toxicity. HSD did result in an increase in plasma sodium concentration compared to the control group given 0.9% saline; however this increase was not excessive. Three animals given HSD after blast did exceed the plasma sodium level of 160 mM associated with chronic toxicity in pigs [33], but not greatly so (none above 170 mM) until the animal was already moribund and HSD had effectively already failed. These levels of sodium are not associated with

sudden death, the problem with sodium levels such as these relate to longer term effects e.g. abnormalities of central nervous system neuronal myelination [33]. Consequently, acute toxicity due to sodium is unlikely to be the cause of HSD failure in the present study.

A further possibility that could not be assessed in the present study relates to the myocardial effect of HSD. Recently the focus on the effects of HSD during resuscitation and been increased in intravascular volumes, microvascular benefit and attenuation of inflammatory responses [19-21; 24; 34]. However, in the older literature an additional effect was postulated: direct augmentation of myocardial contractile force [35]. It is possible that HSD was unable to exert its full effect in blast-injured animals since it is known that blast exposure attenuates myocardial performance [36]. This would not be the case in animals without blast injury, where HSD was found to be especially effective. Unfortunately the data recorded in the present study do not allow a further evaluation of this possibility which therefore remains an interesting proposition for further investigation. A final mechanism that can lead to sudden collapse and heath is hyperkalaemia. As has previously been found [15] blast injury, especially when combined with haemorrhagic shock, can lead to acute elevations in plasma potassium concentration. However, there is no evidence that this was the cause of early demise in the present study since transient hyperkalaemia was a common finding in all of the animals (resuscitated with HSD and 0.9% saline) after primary blast injury and haemorrhage.

Resuscitation with both HSD and 0.9% saline was associated with haemodilution and a fall in arterial oxygen content. This, combined with reduced tissue blood flow due to the shock state (including the attenuated shock state associated with hypotensive resuscitation) resulted in a rise in oxygen extraction ratio (OER) from the resting physiological level of approximately 0.25 to the maximal level of 0.75-0.80. Predictable, blast injury was associated with the highest OER due to the greatest reduction in arterial oxygen content as a result of pulmonary compromise in addition to haemodilution. In all cases the normotensive phase of resuscitation was associated with a fall in OER, presumably because increased tissue perfusion resulted in improved oxygen delivery which became adequate for tissue requirements (ultimately shown by a reversal of metabolic acidosis). In the groups without primary blast injury it was possible to compare HSD and 0.9% saline and no difference in OER was found between the two treatment groups. Therefore from a global (whole body) perspective there was no apparent advantage to HSD. However, this does not exclude a local, microcirculatory, potential advantage for HSD resulting in better blood flow distribution and consequently attenuated metabolic acidosis.

The degree of metabolic acidosis (fall in arterial base excess to negative levels) was significantly less when resuscitation was initiated with HSD in the groups given haemorrhage without primary blast injury. Whether this difference is sufficient to be of profound clinical significance is debatable. Clearly any benefit, especially in a seriously injured casualty, is welcome. However, in the present study the fall in base excess to approximately -10 mM during hypotensive resuscitation, while of some clinical concern, was not overwhelming and easily reversed during the normotensive phase of resuscitation. Therefore the clinical benefit seen in the model used in the present study may not be sufficient to outweigh the potential problems associated with hypernatraemia and risk of overshooting the target blood pressure when HSD is used in austere circumstances. Consequently, a strong case for the adoption of HSD based on the physiological response is not apparent in the present study although there may be circumstances where the logistical burden may drive the balance towards the use of HSD for casualties without blast injury.

In summary, HSD (for initial resuscitation) was inferior to 0.9% saline when haemorrhage was complicated with primary blast injury. This was due to early failure to respond adequately to HSD, or sudden collapse during its use, in a number of cases. By contrast HSD did show a significant physiological benefit when used after haemorrhage in the absence of primary blast injury. However, this benefit may not outweigh the risk of overshooting the target blood pressure during resuscitation especially in austere circumstances where accurate, continuous measurement of arterial blood pressure is impossible. Nonetheless HSD may have a role (in circumstances where the logistical benefit of weight reduction is

very important) for resuscitating casualties where primary blast injury can definitely be excluded e.g. injuries not associated with explosions. Should HSD be used in these circumstances very careful titration of HSD to target blood pressures would be necessary, imposing its own burden on those responsible for resuscitating the casualty under difficult circumstances.

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